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Original article

Retinal thickness analysis with time and spectral-domain Optical Coherence Tomography. Cross-platform interchangeability of manual measurements

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Abstract. *Background and aim of the work:* retinal thickness values obtained by automated analysis with new spectral-domain optical coherence tomography (OCT) devices exceed those measured by old time-domain OCTs. Aim of the present work is to assess reproducibility and comparability of manual measurements performed on both time-domain and spectral-domain OCT scans. *Methods:* 6 eyes were scanned using Stratus OCT3 and Cirrus hd-OCT. Raw exported images were analyzed by ordinary computer software; multiple manual measurements of retinal thickness were taken at different eccentricities. Stratus Retinal Thickness (SRT) was measured from Internal Limiting Membrane (ILM) to the photoreceptors Internal-Outer Segment interface (IS/OS), while two series of measurements were performed in Cirrus images: CRT = Cirrus-RT (from ILM to the Retinal Pigment Epitelium, RPE) and cCRT = corrected-CRT (from ILM to IS/OS). Measurements were repeated twice in two eyes and reproducibility was assessed by Intra-Class correlation (ICC) and Coefficient of Variation (CV). Bland-Altman plots, paired t-test and ICC were used for comparative analysis between Stratus and Cirrus measurements. *Results:* Mean SRT, CRT and cCRT values ± SD were respectively 244.75 ± 34.78 μm, 275.78 ± 34.36 μm and 244.95 ± 33.78 μm. Paired t-test resulted in $p<0.0001$ comparing SRT and CRT series, versus $p=0.6544$ between SRT and cCRT series. ICC was 0.65 between SRT and CRT and 0,94 between SRT-cCRT. Reproducibility of the measurements was excellent (CV=2,13% for Stratus and 1,62% for Cirrus; ICC=0,994 for both devices). *Discussion:* while a systematic error affects comparison of Stratus and Cirrus macular thickness maps, manual linear measurements result interchangeable, hence allowing comparison of images acquired with either OCT systems. (www.actabiomedica.it)

Key words: Time-domain OCT, Spectral-domain OCT, Stratus, Cirrus, Retinal Thickness, Retinal layers, OCT Comparison

Introduction

In middle 90's retinal imaging underwent one of the most striking improvements in its history, with the introduction of Optical Coherence Tomography, which allowed in vivo histology-like visualization of the neuroanatomy of the retina. Stratus OCT1, commercialized by Zeiss Meditech Inc®, represented the first generation of commercially available OCT; it was a time-domain machine, with an axial resolution of 15 mm in tissue and an acquisition rate of 100 A-scan per second. The amelioration of Stratus technology brought to the realization of OCT3, in which both axial-resolution and scan velocity were increased (10 μm, 400 A-scans per second). Recently, a new generation of OCT has been developed and is now available for clinical practice. Spectral-domain OCTs, employing Fourier-based technology, are able to visualize

retinal tissue with a resolution of 3-6 μm on A-scan, and their fast acquisition, up to 30,000 A-scans per second and more, allows to get images of a wider area of the retina and to reduce artefacts due to involuntary movements, hence further improving resolution. Cirrus high-definition OCT (Zeiss Meditech Inc®) is one of the spectral-domain OCTs available today for clinical use and it is characterized by an axial resolution of 5 μm and a transverse sample size of 10 μm on tissue, with an acquisition rate of 27,000 A-scans for second.

Both Stratus and Cirrus OCT systems image retinal anatomy and identify the retinal layers using segmentation algorithms to calculate retinal thickness. Thickness maps are useful in numerous retinal pathologies to quantify macular anomalies and to monitor their changes over time. However, confrontation of thickness values found in healthy and pathological retinas by Stratus and Cirrus OCT has shown discordance, Cirrus resulting in higher values for corresponding retinal sectors (1-7). The underlying reason for this discordance has been attributed to different systems of segmentation: Cirrus, in fact, is programmed to detect the distance between the internal limiting membrane and the outer boundary of the Retinal Pigment Epithelium (RPE) (Cirrus OCT Scanner: Owner's Manual; Carl Zeiss Meditec, 2007), while the external limit of the retina is more superficially localized by the Stratus software, and seems to correspond to the photoreceptors' internal / external segment interface (IS/OS) (1, 4, 7-9). It means that Stratus OCT doesn't consider photoreceptor outer segment as part of the retina while calculating thickness values, thereby introducing a systematic error. If a systematic error in retinal segmentation is actually the cause of discordance between the outputs of Cirrus and Stratus mapping algorithms, overlapping data are expected after correction of the error, e.g. with manual measurements. Any comparison between quantitative measurements requires that linear distances between corresponding points in retinal images by Stratus and Cirrus are equivalent. Linear measurements have been used to quantify retinal layers thickness in conventional and high resolution OCT scans in normal or pathological eyes (10-15) and they are also important as a method to describe characteristics of the retinal lesions visualized by the OCT scan (like the diameter of a macular cyst or dimensions of a RPE detachment).

To our knowledge, comparative studies between manual measurements performed on Stratus and Cirrus OCT scans have never been published. Our purpose was to verify the correspondence between linear measurements in Stratus and Cirrus OCT scans. Identity of linear measurements is meaningful both for the clinical practice, as it would allow quantitative comparison between retinal images obtained with the two devices, and for research purposes, as it would be possible to use cumulative data sets from older and newer technology OCT scans. Fig. 1 shows Stratus and Cirrus scans on the same retina. In this study we performed and compared multiple manual measurements of retinal thickness after correct identification of corresponding retinal layers in OCT images obtained by both Stratus and the Cirrus OCTs on the same eyes.

Methods

The present study was performed with the informed consent of all participants and in accordance with the ethical principles of the Declaration of Helsinki. Six subjects, showing visual acuity of at least 20/40, clear optical media and integrity of retinal layers as detectable by OCT technology, were enrolled; a single experienced operator (A.N.) consecutively examined 1 eye for each patient using two OCT machines, the Stratus OCT3 (software version 5.0) and the Cirrus hd-OCT (software version 2.0), both commercialized by Zeiss Meditech, Inc). Automated signal quality report was used to ensure the good quality of the acquired images. Inadequate scans (score < 6/10) were discarded.

Horizontal, cross-sectional scans of the retina, centred on the fovea (fixation point), were generated by the Stratus OCT using the Line-Scan mode. The resolution was 10 μm (axial) x 20 μm (transverse) and the sampled tissue measured 2 μm in depth x 6 μm in width. Cirrus OCT scans were generated using the '5 Lines Raster' modality, setting the machine to acquire cross-sectional images of the retinal tissue with

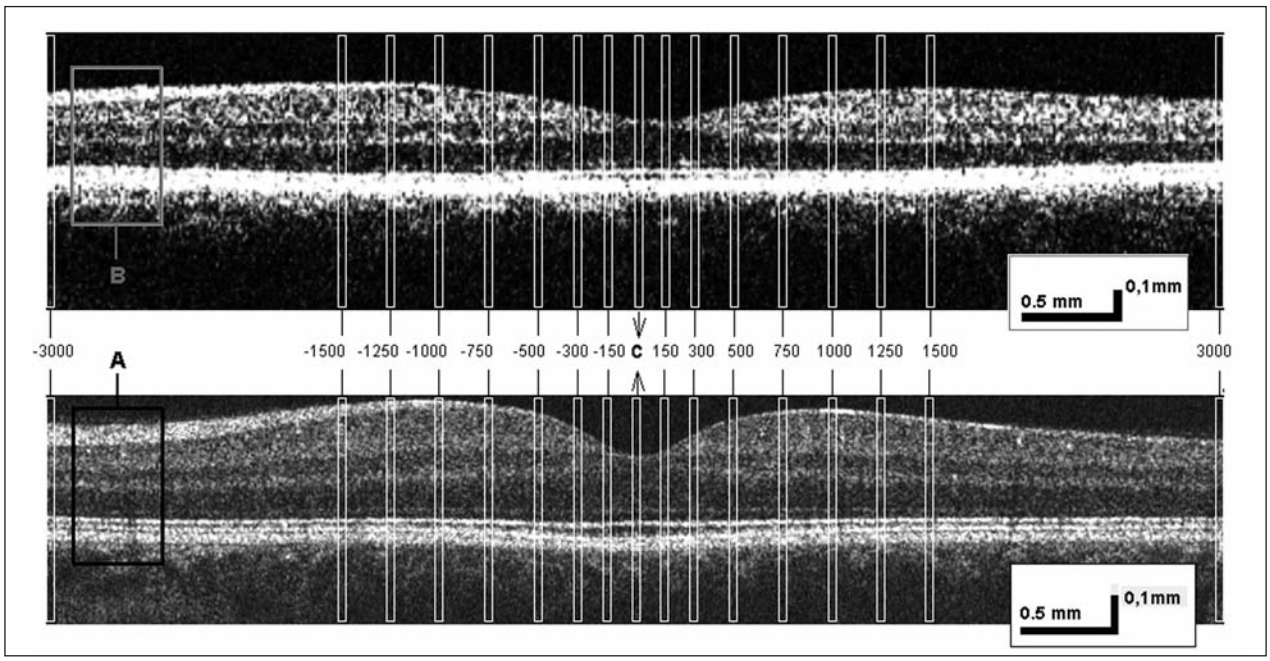

Figure 1. Cross-sectional images of the same retina obtained by horizontal OCT scan centred on the fovea. The image above is by Stratus OCT, the second one is by Cirrus high-definition OCT. White vertical lines indicates the points of measurement. The numbers express the distance (μm) from the centre of the retina. Particulars in rectangles A and B are enlarged in Fig. 2

matching dimension of the Stratus sections (6 mm x 2 mm). The central scan, corresponding to the horizontal meridian, was then selected obtaining a single image with 5 μm axial x 10 μm transverse resolution.

All raw images were exported to uncompressed gray-scale bitmap files (white = maximal reflection, black = absence of reflection) and they were analyzed using the software ImageJ (Rasband, W.S., Image J, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2008). Stratus images were normalized and aligned on RPE plane by the OCT software, Cirrus scans were aligned to RPE after the exportation, by use of a dedicated algorithm of ImageJ, 'Straighten curve objects' (16).

Measurements of retinal thickness were based on a segmentation scheme derived from the literature (10, 11, 15, 17) (Fig. 2). In Stratus OCT images, the ILM was considered the inner limit of the retina and it was identified as the first reflecting plane posterior to the vitreous body. The external limit was placed at the reflecting peak posterior to the Outer Nuclear Layer (ONL) and Internal photoreceptor segment (IS) hyporeflective band (corresponding to IS/OS interface) (Fig. 3a). In Cirrus OCT scans two outer boundaries were considered for alternative measurements, recognized as two reflecting (white) peaks sep-

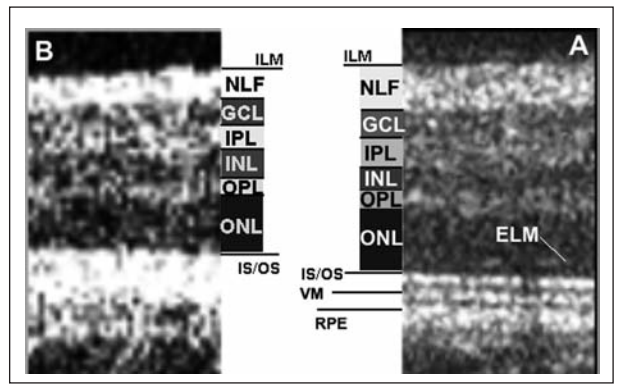

Figure 2. Retinal layers appearance in OCT images (enlarged particulars of Fig.1). B: particular from B-scan image by Stratus OCT. A: particular from B-scan image by Cirrus OCT. ILM = internal limiting membrane, NFL = nervous fibres layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, IS/OS = photoreceptors inner/outer segment interface, VM = Veroheff membrane, RPE = retinal pigment epithelium, ELM = external limiting membrane

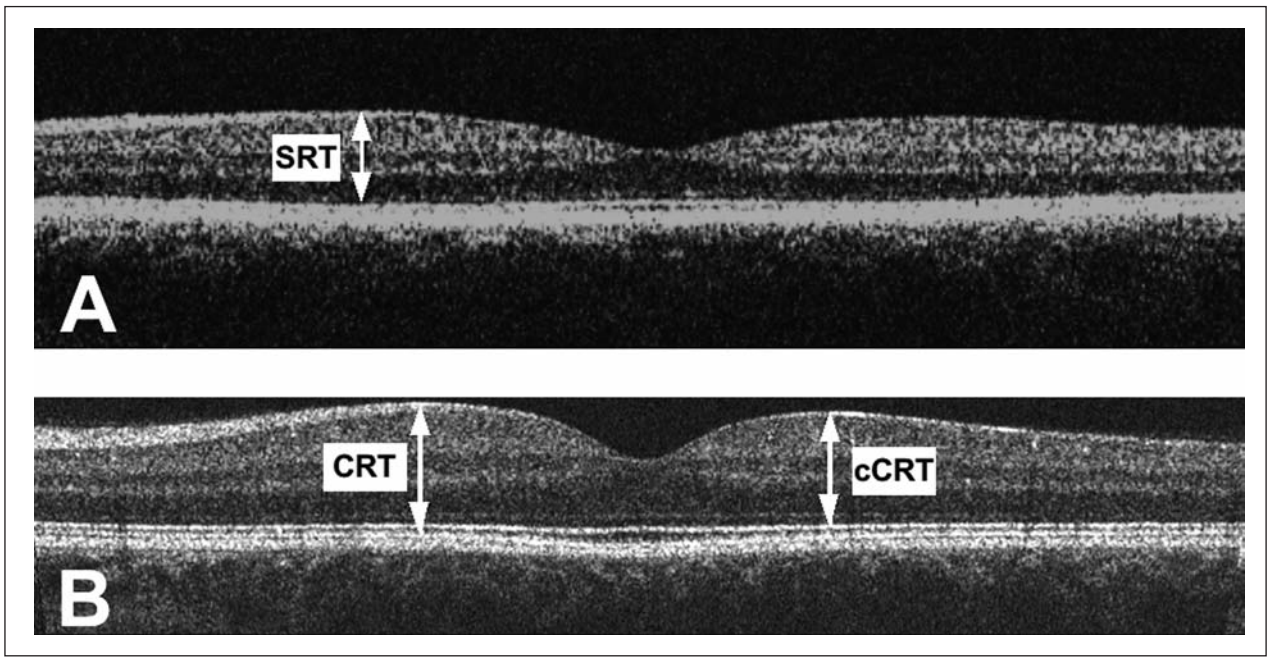

Figure 3. Boundaries considered for the retinal thickness measurement in Stratus (a) and Cirrus (b) OCT images. SRT = Stratus Retinal Thickness. CRT = Cirrus Retinal Thickness, cCRT = corrected CRT = CRT - photoreceptors OS

arated by a thin valley (dark), the first peak just posterior to the largest hyporeflective layer (comprehensive of ONL and IS). The inner line corresponds to IS/OS interface and the outer line to the RPE inner plane (Fig. 3b).

Distance among segmentation lines was measured as a function of retinal eccentricity in 17 points (±3 mm, ±1.5 mm, ±1.25 mm, ±1 mm, ±0.75 mm, ±0.50 mm, ±0.3 mm, ±0.15 mm, foveal centre) using the 'Straight line selections' tool of Imagej (Fig. 1). A single experienced operator performed all measurements in random order. To assess reproducibility, measurements were repeated twice in two images for each OCT system, with a two week interval between the first and the second session to reduce bias due to the operator's memory. We obtained an Intra-Class Correlation (ICC) coefficient of 0.994 for both Stratus and Cirrus datasets and a Coefficient of Variability (CV, calculated as the standard deviation of the differences between the two measurements divided by the overall mean of the measurements) of 2,13% for Stratus and 1,62% for Cirrus.

Statistical analysis was performed with the SPSS software, version 16.0 (SPSS Inc, Chicago, Illinois, USA) and with Microsoft Excel 2003 (Microsoft Corporation, Seattle, Washington, USA).

Agreement between the procedures was evaluated by means of Bland-Altman plots (18) and the Intra-Class Correlation Coefficient (ICC). Significance of the difference between measurements was evaluated with a paired t-test method.

Results

One series of measurements was obtained from Stratus images (SRT, Stratus Retinal Thickness: 17 x 6 eyes = 102 values) (Fig. 3a) and two series from Cirrus images (CRT = Cirrus RT and cCRT = corrected CRT), one for each of the two outer retinal limits we have considered (102 + 102 values) (Fig. 3b). Fig. 4 and 5 show both scatter-plots and Bland-Altman graphs of the comparative analysis between SRT and CRT (A), and between SRT and cCRT (B). While the correlation between SRT and cCRT was excellent (Intraclass Correlation Coefficient 0.94), correlation between SRT and CRT values was lower (ICC 0.65), due to SRT underestimating the measurement.

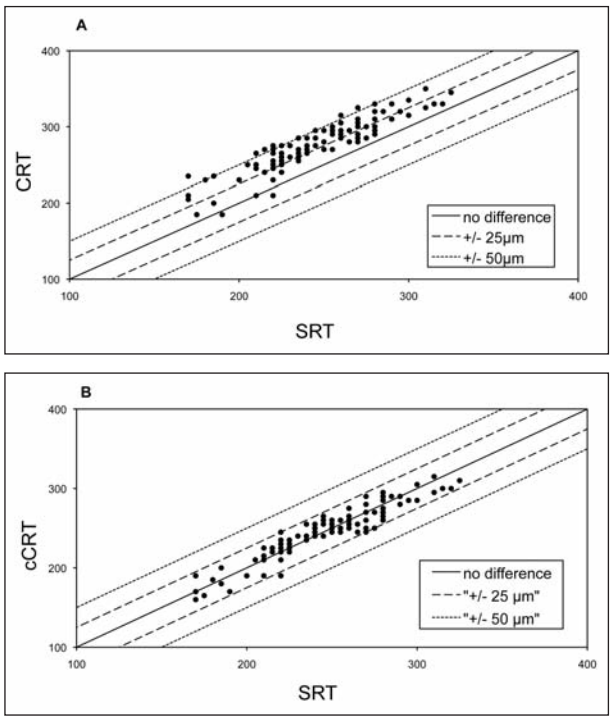

Figure 4. Scatter-plots: A) Comparison between Stratus and Cirrus Retinal Thickness values. (SRT and CRT). A systematic error is present. B) Comparison between Stratus and corrected Cirrus Retinal Thickness. (SRT and cCRT). The systematic error has been corrected

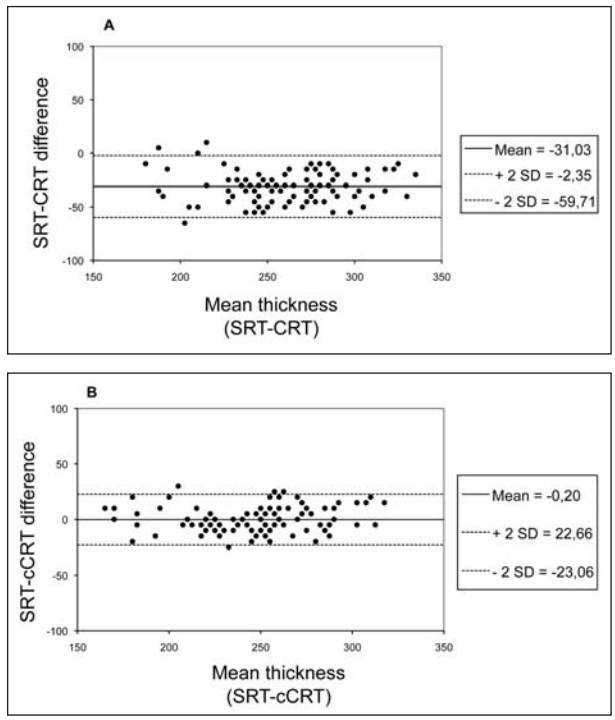

Figure 5. Bland-Altman graphs: A) The thickness difference between Stratus and Cirrus measurements is plotted as function of their mean values. B) The thickness difference between Stratus and corrected Cirrus measurements is plotted as function of their mean values.

Mean SRT, CRT and cCRT values ± Standard Deviation were respectively 244.75 ± 34.78 µm (range 170-325 µm), 275.78 ± 34.36 µm (range 185-350 µm) and 244.95 ± 33.78 µm (range 170-325 µm).

The average difference between paired data of SRT and CRT series was -31.03 µm ± 14.34 (SD) while it was -0.20 µm ± 11.43 (SD) between SRT and cCRT series.

While SRT and CRT values were significantly different ($p<0.0001$, Student's paired t-test), SRT and cCRT series did not show a statistically significant difference ($p=0.65437$). Similarly, OS thicknesses and SRT-CRT difference values were not significantly different at the paired t-test ($p=0.8628$).

The value of CV was 5,5% between SRT and CRT and 4,7% between SRT and cCRT.

Mean SRT-CRT difference was found to vary in relation to retinal eccentricity, being equal to -37 µm at the centre (range -35 to -55 µm), -22 µm (range -10 to -30 µm, $p = 0.05752$) at the nasal periphery and -3 µm temporally (range 0 to -15 µm, $p = 0.00107$), while SRT-cCRT difference didn't show any significant change from centre to periphery and its mean value was null (range -8 to 16 µm, mean = 0 µm).

The OS thickness (CRT-cCRT) ranged between 15 and 50 µm (mean ± Standard Deviation = 30.83 ± 7.71 µm) and it showed a maximum at the central retina, where its mean value was 42.5 µm (SD = 5.24 µm) and a minimum at the temporal periphery (mean value ± SD = 19.2 ± 2.04 µm).

Discussion

Caution is required when comparing OCT images of the retina with real anatomical structures, as optical coherence tomography visualizes proprieties of the tissue that are essentially different from the characteristics that determine the layers' appearance in histological sections. Moreover, quantitative measure-

ment of retinal layers thickness in histological section is hardly uninfluenced by tissue manipulation. Nevertheless, comparative studies in animals confirmed the reliability of an OCT-based interpretation of retinal histology (12, 19) and several studies have been dealing with the identification and quantification of retinal layers in both TD-OCT and SD-OCT scans of human retina (5, 7, 9-11, 13, 14, 20-22).

Previous studies comparing thickness values automatically calculated by Cirrus and Stratus OCT reported mean discordances of 40 to 60 μm (1, 2, 4, 5, 24). The mean difference we found in thickness values between SRT and CRT series was smaller, 31.03 μm ± 14.34 (SD). It is explained by the fact that, as said before, we measured RT from ILM to the VM line, as it was better identifiable in case VM and RPE were partially fused, while the Cirrus software detects a more posterior line (internal to the RPE band).

Basing on the precedent literature, we identified the retinal layers in the OCT scans as shown in figure 2. Verhoeffe membrane (VM) is a thin reflective line that can be seen only in certain high-resolution OCT images among the external layers, just posterior to the connecting cilia line (IS/OS) and anterior to the RPE cell bodies. Marmor et al. (23) proposed that it could represent the zone of the junction of retinal pigment epithelium microvilli and outer segment tips.

In our Cirrus scans VM was often not identifiable, as it was fused with the RPE band, and RPE anterior limit in those cases was not exactly localizable. Therefore, to obtain a homogeneous segmentation, we decided to measure the total retinal thickness (CRT) from ILM to VM when it was present, and from ILM to the anterior limit of the VM-RPE fusion band when isolated VM was not identifiable. Then we measured thickness to the IS/OS (cCRT) to eliminate the difference between Stratus and Cirrus segmentation. In Stratus OCT scans we just measured retinal thickness from ILM to IS/OS. It has been described that in Stratus OCT scans two lines are identifiable at the external limit of the retina: the inner one, giving a stronger signal in OCT, seems to correspond to the IS/OS interface, while the outer, thinner line likely reflects the inner RPE boundary. Automated segmentation by macular mapping software is probably based on the detection of the first, thicker line, as the second one is shallower and often confused with the signal from coriocapillaris (1, 4, 8, 9).

Another point of interest is the zone-related variability in the correlation between thickness measures detected by the two OCTs. Kiernan et al. (4) got a wide range of correlations when comparing average thickness in the 9 sectors of the retinal maps (r = 0.20-0.89). Probably it can be in part attributed to the different pattern of scan acquisition that the two systems utilize for macular mapping function. In fact, Stratus OCT produces topographic maps by obtaining six consecutive cross-sectional scans at equally spaced angular orientations (30°) in a radial spoke pattern centred on the fovea. Cirrus OCT, instead, derives data for thickness calculations from Macular Cube acquisition of 128 linear B-scans x 512 A-scans that are evenly distributed in a 6 mm square centred on the fovea. This way, macula is homogenously sampled, while in Stratus there is a gradient in samples density from a minimum at the periphery to a maximum at the centre, and peripheral values of thickness are therefore far less accurate. By comparison of manual measurements, we found a relatively constant correlation between thickness values (range = 0.74 to 0.99).

An additional factor explaining the variability in correlation constants could be linked to the physiological variation of OS thickness from the periphery to the centre of the retina, where the cone's outer segments become longer and thinner to pack in the zone underling the fovea (25). In fact, we have seen that the OS was thicker at the central retina than at the nasal and temporal periphery (p=0.0001), and a similar profile was found to exist in the difference between SRT and CRT, while SRT – cCRT difference didn't show any significant variation from centre to periphery and its mean value was null.

The main limitation of the present study was the small number of subjects and the absence of automatisms in the analysis procedure. Moreover, the correspondence between the retinal sectors scanned by Stratus and Cirrus OCT was dependent on active fixation of the examined eye, but perfect centring of the fovea provided immediate control of any misalignment.

In conclusion, our results show that the difference between SRT and CRT values can be corrected

when adopting as external retinal boundary in Cirrus scans the IS/OS interface (cCRT), hence confirming that the difference in thickness is due to discordant segmentation algorithms.

Moreover, our results demonstrate that linear distances manually measured on Stratus and Cirrus OCT scans are comparable. This can be useful in everyday clinical practice and in research, as it permits to build cumulative datasets from the two devices.

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